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Prenatal screening for cystic fibrosis: past, present and future

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Prenatal screening for cystic fibrosis is reviewed. The disease, gene involved, molecular basis of disease, genotype/phenotype correlations and pilot trials are discussed, as well as historical perspectives, background and American College of Medical Genetics/American College of Obstetricians and Gynecologists recommendations. A number of complex challenges to the implementation of cystic fibrosis screening exist, including mutation testing of the cystic fibrosis transmembrane conductance regulator gene (*CFTR*), as well as laboratory and clinical issues. Current technologies for *CFTR* testing include reverse dot blots, amplification refractory mutation detection systems, oligonucleotide ligation assays, the Invader[®] assay and NanoChip[®] system. Emerging technologies are also considered, as well as quality assurance measures including analytical and clinical validation, reporting, residual risk calculations and prenatal diagnosis. An even greater challenge is clinical implementation, which focuses upon education and communication, choosing models, reporting, counseling and prenatal diagnosis.

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Historical perspective & background

Cystic fibrosis (CF) has been an obvious target for population carrier screening ever since the gene responsible, cystic fibrosis transmembrane conductance regulator (*CFTR*), was discovered and characterized in 1989 [1,2]. The reasons for this include its high carrier frequency in the general US population (approximately 1 in 25–30 in non-Hispanic Caucasians; progressively less in individuals of Hispanic [1 in 46], African [1 in 65] or Asian [1 in 90] descent, with an overall frequency in the non-Caucasian population of 1 in 56 [3]). CF has a recessive mode of inheritance, with the result that most carriers have a negative family history and do not know they are at risk of having an affected child. It generally has severe (though variable) clinical features, and there is an inability to identify carriers by any clinical or laboratory means other than at the gene level. Furthermore, the implementation of large-scale screening has been hindered by a number of factors, including the size and mutational heterogeneity of the *CFTR* gene, the ethnic heterogeneity of the US population, the technical challenges of developing mutation test panels,

controversies over the clinical burden of the disease, and the anticipated demand and complexity of the associated genetic counseling. This review will describe these various challenges and how they have been met to date, and will provide the authors' perspective on continued evolution and impact of screening programs in the future. For a comprehensive review, the reader is referred to Haddow and coworkers [101] and Richards and Haddow [4].

Cystic fibrosis

CF is usually thought of as a chronic pulmonary disease that may cause progressive, life-threatening respiratory infections. However, it is actually a multisystem disease, with defects in many organs associated with abnormally thick and viscous secretions from epithelial cells obstructing airways and ducts. It is true that chronic obstructive pulmonary disease is the major source of morbidity and early death; however, CF patients may also suffer from pancreatic insufficiency, intestinal obstruction, diabetes, biliary cirrhosis, growth retardation, dehydration due to excessive salt loss in sweat, and sinusitis. In addition, virtually all adult

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males with CF are infertile due to congenital bilateral absence of the vas deferens (CBAVD). With newer antibiotic and other therapies, the median life expectancy has extended to over 30 years, though some patients still die in early childhood from meconium ileus, hyponatremic dehydration, severe protein energy malnutrition or respiratory failure. The pulmonary disease is characterized by obstructive breathing, cough and repeated bacterial infections of the inspissated mucus, with progressive destruction of the parenchyma. Development of pulmonary hypertension can cause secondary right heart failure, so some patients who reach end-stage disease may undergo combined heart and lung transplant rather than lung transplantation alone. With the gene now in hand, there is great hope for an ultimate definitive cure through gene replacement therapy. However, while several pilot studies are ongoing, it is clear that a number of important technical hurdles need to be overcome before this approach can be considered effective on a routine basis. In fact, it is quite possible that this goal may be superseded by other molecular therapies that directly target the gene product rather than the gene itself, such as chloride channel stimulants and CFTR protein modulators. Analogous to many recessive metabolic diseases, the lungs of CF patients are histologically normal at birth, suggesting opportunities for successful pre-emptive or preventative therapies if initiated early enough.

CF is inherited as a true recessive disease, therefore carriers are completely asymptomatic and a child born to two carriers has a one in four risk of being affected.

Cystic fibrosis transmembrane conductance regulator

The CF gene was identified by a tremendous effort involving a combination of positional cloning, gene walking and jumping and candidate gene analysis [1,2]. The deduced amino acid sequence showed striking homology to some known ion channel proteins and indeed the gene product, dubbed cystic fibrosis transmembrane conductance regulator (CFTR), proved to function in that role by transporting sodium and chloride through the cell membrane in an energy-dependent manner. This function was certainly consistent with the pathophysiology of the disease, characterized by abnormal secretions and elevated electrolyte levels in sweat (the basis for the sweat test – the only laboratory method for diagnosis of CF prior to isolation of the gene). The CFTR gene is 230 kb in length, encodes an mRNA of 6500 nucleotides, including 27 exons, and is located on chromosome 7.

Molecular basis of CF

Initial molecular genetic characterization of the CFTR gene in affected patients revealed a recurring mutation, a three-nucleotide mutation of codon 508, which specifies phenylalanine in the protein product and is designated $\Delta F508$ [2]. Within a few months of the gene's discovery, reports from many laboratories began to document a growing number of less common mutations, mostly missense or nonsense but also encompassing deletions and splice-site defects. The catalog has continued to increase ever since, with over 1000 mutations now listed [102].

Most of these are extremely rare or even private (found only in a single family) and thus would not be cost effective to screen for in the general population. However, there are a number of other mutations that recur at an appreciable but far lower frequency than $\Delta F508$. The second most common mutation in the general Caucasian population is G542X (2.4%) [5], and there are another 4–5 above the 1% level. The frequency of the $\Delta F508$ mutation is much less in Hispanic and non-Caucasian populations, unavoidably leading to lower detection frequency of any basic mutation screening panel chosen. In the Ashkenazi Jewish population, the W1282X mutation is remarkably frequent (45% of carriers), improving the screening sensitivity in this ethnic group [6].

Much research is focused on characterizing the precise defect of mutant CFTR proteins. Nonsense and splice-site mutations obviously result in truncated or otherwise abnormally sized protein products, while missense mutations may have a variety of effects on CFTR function. Interestingly, the $\Delta F508$ mutation, representing an in-frame deletion of a single amino acid, does not markedly affect the ion transport function of CFTR; it results in misfolding, preventing a critical post-translational modification step in the endoplasmic reticulum which prevents the protein from reaching its target in the cell membrane [5]. This is intriguing because it may suggest novel protein-targeted therapies for the most common CF defect, even before gene-targeted therapy becomes practical.

Genotype–phenotype correlations

For purposes of biological interest, as well as accurate genetic counseling and rational medical management, it is always hoped that the molecular characterization of a patient's mutation(s) may allow for some reasoned prognostic and therapeutic inferences. Unfortunately, this is not the case for CF, except for a few rather vague generalizations. The early papers described some mutations as being associated with pancreatic insufficiency and others not [7]. For example, $\Delta F508$ is considered a classic, severe mutation causing both lung disease and pancreatic insufficiency, while some other mutations have been associated with lung disease but mild or no pancreatic defect. Beyond that, however, it is highly tenuous to try to predict for a couple the likely degree of severity of their child's disease based solely on identification of the two mutations. Furthermore, there are exceptions to even these basic rules; for example, patients have been reported who are homozygous for $\Delta F508$ but have no lung disease [8]. Meanwhile, there are polymorphisms in the gene that may be harmless by themselves but influence the expression of a mutation on the same or opposite allele (e.g., R117H and 5T).

Pilot trials

Due to the daunting complexities of the CF gene, the inability of any currently available technology to efficiently detect all possible mutations, as well as concern over the nebulous predictive nature of mutation identification, there ensued a great deal of debate within the genetics community over whether such an

imperfect test should be introduced to the public for the purpose of population screening, at least until its utility and impact could be better characterized. To this end, it was recommended that the National Institutes of Health (NIH) fund a series of pilot screening studies to assess the risks and benefits of such a program. The studies were funded through the National Center for Human Genome Research (now the National Human Genome Research Institute) and sponsored by the center's Ethical, Legal and Social Implications (ELSI) program. Five centers were designated to conduct pilot screening programs on general populations with no family history of CF; at least three additional studies were conducted independently of the NIH consortium. The studies varied in population ethnicity, target size, approach (couple-based vs. sequential screening), type of clinic setting, timing of screening (preconception vs. prenatal) and modes of education, counseling and test reporting. By agreement among the centers, screening was largely focused on the six most prevalent CFTR mutations known at the time. Taken in aggregate, the major lessons learned from the pilot studies were:

- CF population carrier screening can be delivered efficiently on a large scale without unduly taxing healthcare providers
- The benefits and risks of CF screening can be conveyed to most patients effectively using a combination of brochures, videos and face-to-face contact with healthcare personnel, but not necessarily requiring certified genetic counselors at all stages of the process
- There was no significant residual anxiety or depression caused by the screening process, even among positive-negative couples
- The process could be incorporated into routine clinical settings in a cost-effective manner
- A number of CF births were prevented in couples who otherwise would never have been alerted to their risk since they had no family history of the disease
- Interest and uptake in screening was appreciably higher when patients and couples were approached in the prenatal clinical setting as opposed to preconceptionally [9,10]

NIH & professional recommendations

In 1997, a consensus conference was held at NIH to evaluate the results of the funded pilot studies and related work by other groups. In light of the generally successful outcomes previously listed, and considering that the best uptake and efficiency was achieved by those studies targeting prenatal settings, it was not too surprising that the consensus panel recommended that CF carrier screening be offered to all pregnant couples and those contemplating pregnancy [11]. This was a general recommendation, without much detail as to which ethnic groups were to be targeted, how many CFTR mutations should be tested, which screening models should be used, how patients and providers should best be educated and counseled and how the various test results should be reported. These issues were subsequently addressed by a follow-up NIH conference [12] and then turned over to a steering committee, composed of representatives from

the American College of Medical Genetics (ACMG), the American College of Obstetricians and Gynecologists (ACOG) and the National Human Genome Research Institute for implementation. This committee in turn appointed several subcommittees to address laboratory testing protocols, patient education and informed consent and provider education.

The products of these working groups have since been published in the form of consensus recommendations and educational brochures for patients and healthcare providers. Model report forms for the various CFTR mutation and polymorphism combinations have also been published for adaptation and use by the testing laboratories [13]. The major recommendations for population-based CF carrier screening are:

- Testing should be pan-ethnic/racial and universal, though perhaps offered more aggressively to the highest risk groups (Caucasians and Ashkenazi Jews)
- Testing should be in the prenatal setting, though preconception screening should be encouraged whenever possible
- Whether testing sequentially or simultaneously, both members of the couple must be provided with their test results
- The minimal core test panel which must be offered consists of 25 mutations and several associated polymorphisms
- The intronic poly-T tract polymorphism is to be assessed only as a reflex test after an individual tests positive for the R117H mutation
- Extended mutation panels beyond the core 25 are not encouraged for general population screening
- Couples with positive screening results or unusual variants should be considered for referral to a genetics center for further counseling [13]

While these recommendations may appear straightforward, when put into practice on a large scale (much larger and including more mutations than the pilot studies), a number of complex challenges inevitably arise. The following sections will address these challenges to CF screening implementation and how they are being met.

CFTR mutation testing: laboratory implementation issues *Technologies*

CFTR mutational analysis utilizes a single nucleotide polymorphism-based technology, of which there are many choices [14]. First, it is important that the laboratory consider a number of factors in choosing a technology, including the ability to support the recommended mutation panel; sufficient (high) throughput with potential for an automated platform; an acceptable (short) turnaround time; a relatively low cost for operation; and a minimal level of technical expertise required to perform analysis.

At the time of this writing, there are no US Food and Drug Administration (FDA)-approved kits for CFTR testing. Laboratories may choose to develop home-brew CFTR tests or use analyte-specific reagents (ASRs), that have been developed by manufacturers for use on several different technical platforms. For those laboratories that choose to develop home-brew assays regulated by the Clinical Laboratory Improvement

Amendments (CLIA) '88 (Federal Register), it is that laboratory's responsibility to collect in-house data to validate test performance. While the FDA does not directly regulate home-brew tests, it does regulate ASRs and these manufacturers must comply with good manufacturing practice (GMP) guidelines. The FDA also requires ASR manufacturers to inform laboratories that analytical and performance characteristics are not established and thus does not allow provision of any information about analytical and clinical validation. Therefore, whether a laboratory chooses home-brew or ASRs, it is the laboratory's responsibility to validate the assay in house. Furthermore, in the USA, the FDA and College of American Pathologists (CAP) require a disclaimer on the laboratory report indicating that the laboratory developed and validated the test and that it has not been approved through the FDA.

The CFTR platforms used by laboratories have changed and will continue to change as new technologies are developed. Based on the results of the ACMG/CAP survey of CFTR testing laboratories in 2000 and 2002 [CAP, UNPUBLISHED OBSERVATIONS], shifts in testing technologies include an increased number of laboratories using the allele-specific oligonucleotide (ASO; forward and reverse) or the oligonucleotide ligation assay (OLA) platforms, some decreased usage of amplification refractory mutation detection system (ARMS) technology, and a significant decrease in use of restriction fragment length polymorphism (RFLP)-based testing (home-brew). Each of these testing technologies will be reviewed in greater depth, although attention will be focused on those for which ASRs are available. The most popular testing methods have been described in ACMG's Technical Standards and Guidelines for CFTR Testing [15,103] and extensively reviewed elsewhere [14]. Here, the current ASR-based platforms for CFTR analysis used in multiple clinical laboratories will be reviewed, including the reverse dot blots Inno-LiPA[®] CFTR33 Probe Array (Innogenetics, Gent, Belgium) and Linear Array CF Gold 1.0 (Roche Diagnostics, Basel, Switzerland), ARMS Elucigene[™] CF29 (Orchid Diagnostics, NJ, USA) and the OLA CF V3.0 (Abbott/Celera, IL, USA). In addition, newly released ASR platforms will be briefly discussed, including the more complex designs of the Invader[®] CFTR (Third Wave, WI, USA) and NanoChip[®] CFTR (Nanogen, CA, USA) technologies. All of these ASRs are designed to identify the ACMG recommended panel of 25 mutations and the reflex tests. More comprehensive mutation-scanning methods (e.g., denaturing gradient gel electrophoresis and single-strand conformational polymorphism) [14], as well as complete gene sequencing [16], are also possible and available in specialized laboratories, but are not applied to routine carrier screening.

The basis of each of these methodologies will be briefly reviewed. The principle of the reverse ASO assay is actually quite similar to that of the forward ASO, as both assays are hybridization based and utilize multiplexed PCR products and ASO probes to the normal and the mutant sequences. In the case of the forward ASO, the PCR products are bound to duplicate filters, each is interrogated using a labeled ASO probe

and results are scored by comparison of hybridization signal to the mutant and wild type [17–20]. In contrast, in the reverse ASO, the wild type and mutant probes are bound to the filter and the biotinylated-labeled PCR product is hybridized and then visualized using a streptavidin–peroxidase conjugate and substrate [21–23]. Presently, the reverse ASO is commercially available in two formats from two different vendors (Linear Array CF Gold and Inno-LiPA CFTR33 Probe Array). Both contain the ACMG-recommended panel, while the Innogenetics strip contains an additional eight mutations. The advantages of the reverse ASO include genotyping, automation capability, nonisotopic detection and medium- to high-throughput capability. The most significant disadvantage for this platform in its current design of combining the mutation panel and the reflex test on a single strip is the difficulty laboratories experience in performing reflex testing according to the ACMG guidelines. Unfortunately, some laboratories have incorrectly chosen to perform (and report) poly-T analysis on all screening specimens tested using this platform, leading to unnecessary confusion over 5T positive results [24].

ARMS technology is based on allele-specific amplification using primers specific to the mutant allele [25–31]. ARMS technology is based on the fact that under stringent conditions, DNA polymerase will only extend if the 3' end of a primer/template hybridization is double stranded. Thus, a mismatch will not allow extension of PCR product. This product utilizes primers specific to the mutation and thus a PCR product results only in the presence of a mutation. This commercially available ASR (Elucigene[™] CF29 ASR) consists of four multiplex reactions using primers specific to the mutant allele and detection requires electrophoresis through agarose containing ethidium bromide, with visualization using ultraviolet transillumination. In addition to the ACMG-recommended panel of 25, four additional mutations are included in this ASR and the poly-T reflex test is packaged separately. An advantage of ARMS ASRs is rapid and easy use with no special equipment requirements. The major disadvantage of the ARMS assay is that the current format does not genotype (i.e., detect the normal allele) in 24 of the 25 CFTR alleles. While this may not be seen as a major drawback in a screening setting in which only heterozygotes are expected, most laboratories use the same testing platforms for both carrier screening and diagnosis; moreover, an occasional homozygote is picked up during carrier screening. In addition, the throughput of this assay is relatively low. Future development of this platform may include a genotyping assay based on the use of multifluorescent primers to distinguish wild type and mutant alleles simultaneously on an automated capillary electrophoresis platform.

The OLA technology (CF V3.0 ASR) consists of two reactions, a multiplex PCR amplification followed by a multiplex OLA, in a single-tube format [32]. The specificity and detection rests within the probe to which the PCR product is ligated. Each allelic probe is specific for the mutant or the wild type sequence and contains a tail of different lengths to allow each to be distinguished upon electrophoresis. A common

probe, which is ligated to the allele-specific probe, contains a fluorescent 3' label to allow detection using an automated sequencer. Data analysis using genotyping software allows automated data interpretation. Principle advantages of the OLA ASR for CFTR include the following characteristics: rapid, easy, single-tube analysis, high-throughput capability on a capillary sequencer, genotyping and automation capability. The major disadvantage is the requirement for an automated sequencer, although most molecular diagnostic laboratories now have this instrument.

Two relatively newer technologies have very recently become available for CFTR platforms and these include the Invader and the NanoChip electronic microarray, which will be briefly described here. The Invader assay is a commonly used platform for less complex assays, such as Factor V Leiden/prothrombin 20210A or hereditary hemochromatosis testing. For CFTR analysis, Invader can be used as a screening platform to genotype for $\Delta F508$ and to screen for pools of less common CFTR mutations in an extremely high-throughput, cost-efficient manner. The technology is based on linear amplification of fluorescence signal generated, rather than exponential amplification of target DNA by PCR. Several probes are used, including a probe to the sequence of interest, a common probe and a fluorescent resonance energy transfer (FRET) cassette. In addition, a proprietary cleavase enzyme is used to recognize the hybridization product, cleave the signal and thus allow the amplification process to occur over time. A series of six reactions are performed, one for $\Delta F508$ and the remaining five for pools of the other 24 CFTR mutations. Results are read with a fluorescence detection system. For pool-positive results, either another method can be employed for cracking the pool to identify which mutation is present, or the Invader technology can be used to break down the pool using an additional set of ASR reagents. The major advantage of the Invader technology is high throughput and low cost, making it an efficient screening tool. The disadvantage is that two to three of 100 tests performed will require additional testing and thus a longer turnaround time. Laboratories that use the Invader technology may also need to have a second platform as back-up for their CFTR testing.

Nanogen technology is basically a forward ASO placed in an electronically controlled microarray format. The current NanoChip consists of 33 mutations, including the ACMG-recommended panel. Each NanoChip consists of 100 test sites (electrodes) attached to platinum wire connections. The negatively charged DNA is electronically guided to a test site where biotinylated samples bind to streptavidin in the site. Following denaturation, fluorescent probes are hybridized to the array and signal is detected after stringent washing procedures. Advantages of this technology include the flexibility of reflex testing, the adaptability of chip design to addition/deletion of mutations in the panel, and the self-contained nature of the constituents. Disadvantages of this platform include the requirement of expensive instrumentation and the high cost of each chip in addition to the ASR reagents.

Emerging technologies with potential application for development of ASRs for CFTR mutation analysis include matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (Sequenom Inc., CA, USA) [33–35] and LightTyper™ (Roche Diagnostics) [36]. The basis of detection by mass spectrometry is molecular weight and alleles are distinguished based on a single nucleotide difference following PCR amplification and a primer extension reaction. There are numerous advantages of MALDI-TOF, including high accuracy in distinguishing base changes, extremely high-throughput capacity, rapid analysis, low run cost and automated platform with data readout. The primary disadvantages are that the instrument cost is significant, the test development is complex and, if designed by the manufacturer, expensive. In contrast, the LightTyper represents an end-point analysis following PCR amplification in a 384-well format and is extremely rapid and high throughput, as well as cost efficient. This technology is based on differential hybridization of a single fluorescent probe to the wild type and mutant sequence, resulting in characteristic melting profiles. In contrast to the mass spectrometry approach, the instrumentation is relatively inexpensive and the test design is rapid and flexible. Limited multiplexing capability is possible in both of these technologies and both are automated and have the potential to interface with the laboratory information management systems software. There are currently no ASRs available for either of these emerging technologies.

Analytical & clinical validation

The test validation process is focused on a collection of data to establish the analytical validity, the clinical validity and the clinical utility of the test. For a more extensive discussion, the reader is referred to the ACMG Standards and Guidelines for Clinical Genetics Laboratories [103]. The validation process consists of the following steps: review professional guidelines and relevant literature; evaluate the analytical performance within the laboratory; define the test limits; monitor test variables that affect performance; identify the ethical, legal and social issues surrounding the test; and assess known information about the clinical utility of the test. A number of guidelines and regulations are available to help laboratories in the validation process [37,38,103–105]. Each test must be validated for preanalytical, analytical and postanalytical components. Preanalytical validation encompasses patient and provider education, collection of patient information and consent, and specimen issues including collection, transportation, processing and storage. Analytical validation includes assessment of the analytical performance of the test, with determination of the test's analytical sensitivity and specificity [39]. Regardless of the technology used, ASR or home-brew, the laboratory must perform an in-house analytical validation. The laboratory must also determine the clinical validity of the test, either in-house or through literature review, and document the clinical sensitivity and specificity for the intended use of the test. An important component of clinical utility of the CFTR test is determination of the clinical sensitivity (mutation detection frequency) and birth prevalence based on

race/ethnicity. The laboratory will also be required to document the positive and negative predictive value of the test and genetic, environmental or other modifying factors that may affect the test result. Postanalytical validation comprises reporting the results, education of and communication with providers, follow-up on results, addressing ELSI issues when present and providing an information source derived from the literature for determining the clinical utility of testing. Such information may include the risks and benefits of testing, results of pilot trials, quality assurance information, interventions for specific outcomes in testing and financial costs and economic benefits of testing.

Reporting & residual risk

Clinical laboratory reports for CF carrier screening will in most cases be either positive (heterozygous for a CF mutation) or negative for the mutations tested and a residual risk will be given. However, there will be exceptions, such as individuals with mild symptoms of CF found to carry two CF mutations, or identification of CBAVD-associated mutations. A positive test result in which an individual is found to carry a CF-causing mutation will be considered first. These reports are relatively straightforward: identify the mutation and the patient as a heterozygote carrier and recommend testing the partner. Negative results require an estimate of the individual's residual risk for being a carrier and this risk is based on the family history of CF, the patient's ethnicity and the mutation detection frequency. In the case of CF carrier screening in the general reproductive population, most individuals tested will have a negative family history of CF. For individuals with a positive family history, a prior risk of being a carrier should be determined by a geneticist and used in the residual risk calculation. In all cases, it is important to determine the patient's ethnicity/race, as both the CF gene frequency and the mutation detection frequency differs among these groups. The ACMG Standards and Guidelines provide updated residual risk tables based on ethnicity for individuals with no family history of CF, which are useful to include in negative reports [15,40,103]. Model report formats have also been provided for laboratories by ACMG [13]. Also considered in these models are the complex reporting situations with certain CFTR mutations, particularly R117H and 5T, and the reader is referred to these resources for a more detailed discussion. Since the mutation panel is evolving and modifications are anticipated in the near future [24], it is likely that the reporting formats will also need to remain fairly fluid.

While most laboratories report the individual's test result, physicians screening reproductive couples to identify a CF fetus and some laboratories using this programmatic approach will focus on the couple's result. Depending upon the model used in testing, a couple's report may vary. For example, if a sequential screening model is used and the first partner tests negative, then the second partner would not be tested (case 1). The couple's residual risk to have a child affected with CF (assuming no family history and Caucasian ethnicity in this and all following examples) would be calculated as follows:

- $1/200$ (first partner, negative) \times $1/25$ (second partner, untested) \times $1/4 = 1/20000$

In contrast, using a concurrent approach, both are tested and if both are negative their residual risk is lowered as follows:

- $1/200$ (first partner, negative) \times $1/200$ (second partner negative) \times $1/4 = 1/160,000$

In both cases, no further testing or genetic counseling is recommended. An issue here is who is responsible for co-ordinating these results? The laboratory or the physician? The laboratory is fully capable of performing the necessary calculations, however, in many cases, both patients are not sent to the same testing laboratory. More importantly, however, are the new Health Insurance Portability and Accounting Act rules of patient confidentiality, and it is not entirely clear whether the laboratory based in the USA is at liberty to combine couples' results. From the physician's perspective, it may be desirable that laboratories do report couples' results as it removes the burden of genetic calculations from the already overburdened obstetrical practice. At the current time, we do not have all the answers to best practice guidelines for laboratories and physicians performing CF carrier screening in the reproductive setting. However, as with all new programs, in due time these important issues will evolve in a positive direction.

On the other hand, if the first individual tested is found to be positive, then the partner is tested. In this case, there are two possible outcomes (actually more if one considers the CBAVD issue, however, this discussion will be limited to the obvious ones). First, the partner could be found to be negative, resulting in what has come to be known as the positive-negative couple. The residual calculation for these couples is as follows:

- 1 (she is a carrier) \times $1/200$ (his test is negative) \times $1/4 = 1/800$ to have a child with CF

While these couples are at increased risk over the general untested population, they are not suitable candidates for prenatal diagnosis and it is not recommended, nor is additional testing for more mutations in a larger panel encouraged [13,41]. There has been concern expressed regarding increased anxiety of these couples; however, the pilot trial data have shown that in fact, these couples do not experience this projected anxiety [9,42].

On the other hand, another outcome for testing the partner of a CF carrier is that he too could be found to be a carrier. In this case, the couple has a one in four risk for having a child affected with CF and genetic counseling is recommended and prenatal diagnosis is offered. In these positive-positive couples, genetic counseling is focused on providing information regarding prenatal diagnostic options for the current pregnancy and our current understanding of the disease, treatment and prognosis. In all cases in which an individual is positive for a CFTR mutation, genetic counseling is recommended and testing is appropriate for at-risk family members and the CF carrier is provided with materials to share with these family members who may wish to consider testing.

A different approach to screening is taken by some laboratories that perform couple screening using the Wald approach [43]. In all cases, samples from both reproductive partners are collected. However, only the female sample is tested. If she is found to be negative, no further testing is performed. If she is found to be positive, the partner's sample is tested. Reports are written for the couple, not the individual. A positive report for the couple requires that both are positive for a CF-causing mutation. In the case of the positive-negative couple, the couple is given a negative result, from which they may infer that they are not at increased risk for having a child affected with CF. Although this is not strictly correct, it is accepted under the rationale of the model since no further intervention in the pregnancy is indicated. This model, which is a screener's approach, has generated some controversy in the genetics community in the USA. Both the positive and negative aspects of this model will be briefly reviewed. The positive features of this model include higher efficiency and lower cost, which are desirable features of a screening program. In addition, this model eliminates any concern over increased anxiety of positive-negative couples because they will never know their individual results and that they are in fact at increased risk over the general untested population. The controversy is based on concern from geneticists that this model eliminates the positive benefits of cascade testing (i.e., alerting at-risk family members of the identified CF carrier [44]) and that it sequesters the information that an individual is in fact a CF carrier.

Current problematic issues in CF reporting

Most of the mutations on the ACMG-recommended panel actually cause CF and the reporting of results is relatively straightforward, as previously described. Even so, genotype-phenotype correlations are too variable to be used clinically and overly specific prognostic reporting is discouraged. There are two exceptional mutations on the panel that may or may not lead to a CF phenotype and their expression is dependent on modifiers. One is the R117H mutation, which can be a CF mutation in the presence of a 5T allele on the same chromosome (*cis*) or a CBAVD-associated mutation when in *cis* with a 7T allele. Comparison of R117H frequency in the general population versus the CF population indicates that it occurs around 20-times more frequently in the general population than predicted, which suggests that it occurs in two different forms. The 5T allele is very common in the general population, occurring at a frequency of approximately 5% in Caucasians and with no phenotype in most carrier individuals (though homozygous 5T/5T males may have CBAVD). Both CF and the CF-associated disorder CBAVD are recessive disorders and thus clinical phenotype occurs only in the presence of two disease-causing alleles. Thus, the ACMG recommended that the R117H mutation be included in the screening panel (since it does cause CF) and when identified, that reflex testing for the 5T allele be performed as a routine laboratory procedure to enable more informative genetic counseling. Unfortunately, some laboratories have not followed this guideline and instead

routinely test for and report results of the 5T analysis on the first pass [24]. This has led to the unfortunate situation of unnecessary anxiety and anecdotal reports of prenatal testing being performed for 5T carrier couples, even though this condition does not lead to a clinical phenotype of CF. Some laboratories have left the decision of whether to perform 5T screening to uneducated providers who assume that more testing is better. This case represents an excellent example illustrating how more can in fact be harmful and counterproductive to the central issue.

The second example of a mutation with variable expression depending on haplotype is I148T. When this program was initiated, our committee that recommended the mutation panel had no precedent to follow in making the panel selection. As our cut-off criterion, a mutation frequency of 0.1% or greater in the general (mixed ethnicity/race) US affected CF population was chosen, as catalogued in the Cystic Fibrosis Foundation Registry of Genotyped Patients [45]. Using that standard, I148T had been reported by the CF registry at just over 0.1%. Within a relatively short period following screening (<1 year), reports from large testing laboratories surfaced indicating that I148T was identified almost 100-times more frequently in the general population as compared with the CF population, a story all-too familiar at this point [46,47]. Rolhfs and coworkers identified a genetic modifier, a deletion of six base pairs (3199del6), resulting in an in-frame deletion of two amino acids in the CFTR protein that traveled with I148T when it was a CF disease-causing allele [46]. No CF patients were identified with I148T that did not also have 3199del6. The questions then became: is 3199del6 the real CF mutation and I148T simply a polymorphism in linkage disequilibrium; does 3199del6 alone cause CF and is it ever found unlinked; or can it also be linked with other CFTR mutations? These questions raise several issues. One illustrates the utility of good pilot trials. Screening for only a small number of mutations (with lower detection rates) could have been chosen, or a large pilot trial with all 25 mutations could have been demanded before initiating a national population screening program for all reproductive couples. However, the authors argue that either of those decisions would have been the wrong choice at the time. The increase in detection rate from approximately 80% with the six-mutation panel used in the initial pilot studies [48] to close to 90% [13], with little increased laboratory burden given the available technologies, was seen as desirable and there would have been a loss rather than a gain by dropping numbers of mutations tested; this was simply not an option. And neither was the second option as funding, initiation and evaluation of a larger screening program for 25 mutations would have greatly delayed this long-overdue program. Others have argued that with current technology, many additional mutations, including those specific to particular minority racial groups, could have been included in the core panel [3]. Yet the overriding goal was to develop a minimal, pan-ethnic screening panel that would comprise the best characterized mutations and be technically and economically within the means of most laboratories. A

danger of expanding to more rare and less studied mutations is that their clinical significance may be questionable, as has already been experienced with I148T.

In conclusion, we can learn from these anomalies. One lesson is that good data collection is imperative to the success of this program. In a sense, this is analogous to postmarket surveillance conducted by the FDA on a new prescription drug, even after it has passed pilot-phase clinical trials. ACMG has proposed this initiative, although has not provided a detailed mechanism for its development, and efforts are underway at the Centers for Disease Control and Prevention (CDC) to move this important component forward. The committee to review the panel of mutations has reconvened and is currently deliberating the merits of inclusion or exclusion of the R117H, 5T, I148T and 3199del6 mutations/variants.

Quality assurance

Quality assurance is the program that laboratories develop to ensure high-quality and clinically useful results [101]. Clinical laboratories must follow good laboratory practice, including external proficiency testing. While general overarching guidelines for molecular genetics laboratories exist (ACMG Standards and Guidelines, CAP Checklists, Federal Register CLIA '88, National Committee for Clinical Laboratory Standards Molecular Methods and New York State Department of Health), there are now specific guidelines for CFTR testing and the reader is referred to these for a more detailed review [15,101]. All US laboratories performing CF testing must comply with CLIA '88 guidelines. In addition, US laboratories that maintain certification by New York State or CAP must also comply with these respective guidelines, which are often at a higher level than the federal guidelines. The checklists provided in such guidelines include quality control, quality assurance and more recently quality improvement, and they are also a useful resource for laboratories in order to establish high levels of excellence. Sections in the CAP molecular pathology checklist include: extent of services, methodology, quality control, reagents, equipment, physical facilities, specimen handling, procedure manuals, procedures and tests, controls and standards, requisitions and reports, records, proficiency testing, laboratory safety, laboratory management and test validation [104]. Proficiency testing for CFTR mutation detection has long been available in both the USA [49] and Europe, which has its own quality assurance program [50].

There are unique quality assurance considerations for CF screening. An important preanalytical consideration is obtaining appropriate patient information, including family history of CF (and if positive, relationship to affected and whether CF mutation testing was performed and mutation(s) identified), ethnic background, gestational age of fetus (when appropriate) and carrier status of partner (if known). It is of paramount importance to identify whether the test being ordered is for CF carrier screening or whether it is diagnostic (and if so, whether it is a differential or definite diagnosis). In cases of prenatal testing, it is important for the laboratory to obtain parental

samples for reconfirmation and for maternal cell contamination studies (and previous reports if CF carrier testing was performed in another laboratory), gestational age of fetus, type of fetal sample and results of cytogenetic analysis (male or female). An issue of significant importance and debate is the requirement of informed consent. The ACMG guidelines, ACOG guidelines and New York State guidelines all indicate that informed consent is necessary for CF screening. The physician is responsible for consenting the patient, either verbally or written. The laboratory is responsible for providing the physician with the necessary educational materials (including a consent form) to allow the physician to educate the patient and administer informed consent. Many laboratories include a space on their test requisition for the physician to attest to the compliance of the informed consent process with this patient. This item is still in evolution at a professional society level and we may expect to see remodeling in the future. An additional part of the informed consent process may include the consent of the patient for the reuse of specimens. The reuse of patient specimens, depending on purpose, has many restrictions. For example, if reuse is for research purposes, an Institutional Review Board approval is required and usually a new consent, except in some cases where the samples are anonymized. A broader interpretation may be given for use of samples for quality control/quality assurance purposes, although new federal regulations may preclude even this application. Laboratories are encouraged to be aware of local and national regulations with respect to storage of and reuse of clinical specimens. This issue impinges directly on the difficulty of procuring positive mutation controls.

Analytical issues include determination of the analytical sensitivity and specificity for the CFTR mutation technology platform chosen for use by the testing laboratory. Currently, there is no direct comparison among all of the known CFTR testing technologies, including the home-brew and the various ASR-based methodologies. There is a need for such a comparison. However, the experience to date, both in the field and in nationwide proficiency testing programs, suggests that all the commonly used methods are extremely robust, with very low to negligible false-negative and false-positive rates [39]. An equally important analytical consideration is the lack of available positive controls of all 25 CFTR mutations. While the Coriell Institute (NJ, USA) has the majority available, there are several which are not included in this repository. The CDC has funded two projects to develop natural and synthetic mutation controls that may be provided to clinical testing laboratories [51,52], although these are not currently available. An issue that both ACMG and CAP have addressed is the need for inclusion of positive controls on each CF test run, currently implied by strict interpretation of the CLIA regulations. While it is desirable to run all mutation controls on each run, it is recognized that this is not feasible with all technologies and would entail an unrealistic cost and labor burden, especially if we were to move on to even higher density mutation arrays. Thus, both groups have recommended that the laboratory develop a rotating

system for the less common mutations, while running the common mutation controls ($\Delta F508$) on each run. As a deemed laboratory accreditation agency under CLIA in the USA, the CAP proposal is under review for compatibility with the CLIA existing regulations at time of this writing.

Prenatal diagnosis of CF

The goal of the prenatal screening program is to identify fetuses affected with CF, which is ultimately accomplished through prenatal diagnosis. When is prenatal diagnosis recommended? Prenatal diagnosis is indicated for couples in which both partners are carriers of CFTR mutations and thus the fetus is at one in four risk of being affected. Thus, it is important to perform carrier testing early in pregnancy in order to identify such at-risk couples in sufficient time (before 20 weeks of gestation) to allow the option of prenatal diagnosis and possible termination of a fetus. An additional indication for prenatal testing is echogenic bowel of the fetus found on ultrasound in the second trimester. Echogenic bowel is found in approximately 0.1% of pregnancies in the second trimester and can be due to CF, normal variation, chromosomal abnormality or congenital viral infection. In these cases, the risk due to CF is estimated in the range of 3 to 13% [15]. The finding of two CFTR mutations confirms the diagnosis of CF, whereas one increases the risk of CF to 13–43% and the finding of no mutation decreases the risk to less than one in 650 (assuming a Caucasian fetus).

Prenatal specimen types include both direct and cultured amniocytes and chorionic villi. Chorionic villus sampling (CVS) is performed during the first trimester at approximately 10 weeks, while amniocentesis is performed in the second trimester at around week 16. Both are invasive procedures and carry a small risk of fetal loss (approximately 1%). In addition to necessary fetal and parental specimen requirements (including both type and amount), there are special information requirements that ensure quality analysis of prenatal specimens. These include an estimation of the gestational age of the fetus, fetal sex (based on the cytogenetic analysis results) and information about the parents' CF test results. It is good laboratory practice to test the parents of the fetus in the same laboratory that the fetal specimen is tested, to ensure appropriate interpretation of results, and to perform additional laboratory tests in order to rule out maternal specimen contamination.

Maternal cell contamination (MCC), a term used to describe the contamination of direct and cultured amniotic fluid or CVS with maternal cells, is a source of error in prenatal diagnosis. Based on cytogenetic data, MCC occurs at a frequency of 0.6–1.0% in cultured amniocytes, 0.1–0.9% in direct CVS and 1.8 to as high as 12.6% in cultured CVS [101]. MCC can be evaluated in virtually 100% of cases by comparing the fetal and maternal DNA specimens using a combination of several polymorphic short tandem repeat or variable number tandem repeat markers. Ideally, a paternal specimen will not be required for determination of MCC, although in some cases this may be necessary. It is important

for laboratories to determine how their testing methods are affected by the presence and amount of MCC and the validation study can be performed using DNA mixing studies as recommended in the ACMG Standards and Guidelines for prenatal diagnosis [103].

Prenatal testing should not be performed to determine the 5T status of the fetus. The 5T allele in the absence of a linked CF mutation does not result in a clinical CF phenotype. Laboratories routinely testing for 5T in a prenatal population are not following the ACMG guidelines and are creating needless prenatal testing for 5T-positive couples. Genetic counselors are asking for better guidance on how to counsel individuals and couples positive for the 5T allele. Testing for the 5T allele has generated significant confusion for both laboratories and providers in the aftermath of the ACOG/ACMG recommendations. Some laboratories that have included 5T in routine testing due to technological constraints have chosen simply not to report the results and while there is some merit to this practice, there is also some debate surrounding whether such practice creates an additional liability issue for the laboratory. As with any new initiative in its infancy, we are now in the process of uncovering the pitfalls and addressing quality improvement. Clearly, more education is required for both the laboratory and the provider regarding appropriate testing and interpretation.

Clinical implementation issues

Education

It was recognized early on in the pilot studies that the success or failure of such a complex genetic screening program would rest primarily on the effectiveness of information transfer between physicians, patients and testing laboratories. The pilot studies demonstrated that this could be accomplished with well-constructed printed or videotaped materials. Through the work of the steering committee groups, patient educational materials have been produced and distributed in the form of two brochures: *Cystic Fibrosis Carrier Testing: The Decision is Yours* and *Cystic Fibrosis Testing: What Happens if Both My Partner and I are Carriers?* Similarly, an excellent booklet for provider education has been prepared by ACOG and distributed to its members, entitled *Preconception and Prenatal Carrier Screening for Cystic Fibrosis*. The laboratory community has meanwhile been informed by ACMG as to the recommended techniques and approaches for performing CF mutation screening [15,24].

Models

Much deliberation went into the decisions regarding recommended models for delivering the screening product to patients. The desire was to keep these recommendations as broad as possible, recognizing that widely divergent patient populations and clinic situations will require different approaches. While admitting that preconception screening is emotionally preferable to testing during pregnancy because of the increased range of options that can be offered to carrier

couples identified early, the steering committee recognized that both the CF pilot screening studies and earlier genetic screening programs had shown that uptake is much greater once couples are already pregnant. That is the reason the ACMG/ACOG recommendations are referred to as prenatal CF screening, even though we continue to encourage physicians to suggest preconception screening whenever feasible. Regarding sequential versus couple screening, the committee took no preferential position, with the proviso that, whichever model is chosen, results must be communicated in full to every individual tested [13]. This recommendation effectively excludes the couple-based model of Wald [43], for reasons already discussed.

Reporting/counseling

Prior to the publication of the ACMG/ACOG recommendations, very few of the approximately 50 CF testing laboratories in the USA were screening for more than 14 CFTR mutations [49]. In order to bring the laboratories up to speed on reporting for this larger and more complex panel, the ACMG recommendations included an appendix containing several model test report forms representing the major positive and negative outcomes, along with various combinations of the R117H mutation and 5T/7T polymorphisms [13]. Laboratories are not obligated to reproduce these forms word for word but they serve as a basis for inclusion of the key facts in their own reports. Similarly, the information conveyed in the forms can be used by primary care physicians or genetic counselors for post-test result interpretation and counseling. It is suggested that the following test result situations (among others) should prompt consideration for referral of the couple to a specialized genetics center for more lengthy and target counseling: positive-positive couples, positive-negative couples with residual anxiety, individuals with a family history of CF, individuals testing positive for R117H and the 5T/7T polymorphism, and infertile males who are found to carry a CFTR mutation or variant. However, it must be acknowledged that discussion, even with some negative-negative couples, of all the general issues of pre- and post-test counseling can be quite time consuming and complex. This undoubtedly accounts for some of the resistance or trepidation that some obstetricians feel about integrating this program into a busy office practice.

Prenatal diagnosis

The option to proceed with prenatal diagnosis will naturally be raised with any couple that tests positive-positive for any of the 25 mutations in the panel. The technical considerations that arise during CFTR mutation testing on the fetus have already been discussed. For counseling aspects, the couple is arguably best served by referral to a genetic counselor to go over in detail the options available. If the couple is already pregnant, the options are to do nothing, do nothing now but test the baby when born, or do prenatal testing by either amniocentesis or CVS and terminate a fetus found to carry both parental CFTR

mutations. If the couple is not yet pregnant, other options are possible, such as *in vitro* fertilization with preimplantation genetic diagnosis, artificial insemination with an unrelated sperm donor (who has first been tested for his own CF carrier status), or simply opting not to have children. It should also be noted that some couples that proceed with prenatal testing later decide they cannot face pregnancy termination even if the fetus is found to be affected. Ideally, it would be best to assess such attitudes early on so that the fetus is not exposed to the risks of prenatal sampling unnecessarily, but this is not always possible. Some may argue that the information is worth having so that couples will know what to expect at time of birth (analogous to the rationale behind newborn screening programs for CF); however, such arguments are not considered scientifically compelling.

Expert opinion

The recommendation for CF carrier screening in the reproductive population has had a significant impact on laboratories, physicians, counselors and ASR manufacturers/technology developers. Both academic and commercial laboratories performing CF testing have experienced up to fivefold increases in test volume since the recommendations were put in practice. Given this increase in volume, along with the knowledge of significant noncompliance with these guidelines in practice (i.e., not offering testing to patients), it is anticipated that the testing volume (compliance) will continue to swing upward, with the possibility of a tenfold increase in volume. With such increased volume comes concern for a careful monitoring (both internal and external) of laboratories' quality assurance/quality improvement programs in order to ensure a high standard of excellence. Moreover, this begs the question of how much can laboratories scale-up for this testing and will they reach a point of saturation, even in the larger commercial laboratories? With the amazing improvements in technology, the authors predict that laboratories will indeed be able to meet this challenge with relative ease; however, they will need to be proactive. One issue which has surfaced is that large testing laboratories may be perfectly content with carrier screening but may wish to farm out their prenatal diagnostic testing, which is inherently of higher liability, costs more to perform and is generally a financial loser. We may see a trend for boutique prenatal testing laboratories that price testing at a premium to cover insurance costs and the multiple reconfirmations necessary for these analyses. It is also expected that more laboratories will initiate CF testing, particularly since there is a high volume of tests in the market, ASRs are commercially available with relatively simple testing platforms, and there is currently an overabundance of qualified clinical molecular genetics laboratory directors that can perform and interpret these tests.

It is predicted that physicians, particularly obstetricians but also family practitioners, will become educated and compliant with the ACOG recommendations and thus generate more CF test requests. This change in standard of practice will be

partially influenced by the pending lawsuits that allege not offering CF testing to a pregnant couple whose baby now has CF. This will likewise push insurance carriers to pay for the testing more uniformly than is occurring now. Unfortunately, practice in the USA is often driven by litigation and CF screening is no exception.

The authors predict that counselors will develop standard guidelines for CF counseling that can be utilized by nurse practitioners, as well as physicians. While comprehensive educational materials have been provided by ACMG and ACOG, counselors may be most effective by providing in-service training for referring practices in order to educate the educator. It is anticipated that counseling practices will expand based on demand for this test in particular and that multiple avenues of counseling will become accessible through appropriate training. At the time of writing there is significant confusion even among certified genetic counselors on how to counsel when the 5T variant is detected. Soon, this may not be a counseling problem because laboratories will either not test for it routinely or not report it.

The expansion of testing technologies will continue. We can anticipate great success for the ASR developers and new platform developers, with more technologies in the future. More efficient technologies will also lower the cost, making insurance or governmental coverage easier. This industry is not saturated and continues to evolve and improve rapidly.

Less clear is when, if ever, the ethical issues surrounding CF screening will be resolved. There will always be those who consider the disease too variable in severity, or sufficiently amenable to treatment, to justify the massive effort and expense of population carrier screening and resultant pregnancy terminations. This perception may even grow more fixed as novel gene-specific therapies emerge. At this point, however, the program offers couples potentially useful information about their genetic risk and the opportunity to use this knowledge to optimize their reproductive outcomes.

Five-year view

At this point, nationwide CF carrier screening is still in its early stages. While a number of successes and unexpected problems have emerged, it is yet too soon to tell whether the program will ultimately achieve its goals, the primary one being to reduce the number of unwanted CF births. To date, the best evidence that this may be happening comes from the rather controlled and self-contained Health Maintenance Organization population managed by Kaiser-Permanente in northern California [42,53]. In the rest of the country, we are still dealing with fairly low participation in the screening program by obstetricians and family practitioners. This may improve as test costs (currently in the US\$120 to US\$280 range) begin to diminish with the advent of more high-throughput technologies and as practitioners become convinced of the cost effectiveness of the program as already demonstrated at Kaiser-Permanente. Considering that offering the CF screen to all pregnant couples is now deemed standard-of-care by their own professional organization

[54], it is probably only a matter of time before one or more lawsuits over wrongful life are lodged against physicians who failed to offer the test to their pregnant patients, even in the absence of any family history of CF (if such has not happened already). Undoubtedly, the first defendant loss in such a case will provide a huge stimulus for the remainder of the obstetrics community to get on board.

From the laboratory standpoint, the recent availability of robust ASR methods targeted at the recommended 25-mutation panel has made life easier and will likely encourage more laboratories to get into the CF testing field. Some of the newer ASRs may become so user-friendly that they require little or no expertise in molecular biology to perform. In this case and since CF testing has become high volume, there will be a temptation in some centers to move this test into the high-volume clinical chemistry laboratory, where it may become divorced from needed clinical genetics expertise. This, in our opinion, would be an invitation for trouble. As pointed out earlier, the analytical portion of a molecular genetic test is just one component of the testing process; the pre- and postanalytical portions are just as important and carry just as much risk for harm to patients if not conducted thoughtfully and carefully.

Lastly, the core mutation panel, currently consisting of 25 mutations and associated polymorphisms, is under constant re-examination by the ACMG committee and will undoubtedly change in the coming years. The first changes are likely to be minor, perhaps involving the I148T or R117H mutations. But as testing technology becomes even more efficient and cost effective, it will become possible to screen for an ever-greater number of mutations. In fact, it may be possible within 5–10 years to perform complete sequencing of the CFTR gene at a cost that is low enough to be used for screening (some laboratories already offer complete sequencing but the cost is prohibitive for screening, so the test is reserved for diagnostic purposes). While this prospect may appear tempting as a means to increase screening sensitivity, especially in non-Caucasian populations, we must remember the potential downsides of such an approach. For one, complete sequencing will reveal variations and polymorphisms that have never been reported before and their clinical implications for purposes of genetic counseling will be impossible to determine. Second, we must remember that as analytes (mutations) become increasingly rare in the target population, the likelihood that a positive result is really a false positive also increases. These factors should compel us to be cautious in demanding ever-higher numbers of mutations in the core screening panel. This is, after all, just a screening test and patients are warned at the start that not every carrier will be identified. Compared with what couples with negative family history were offered before (i.e., nothing), one could argue that 25 mutations are perfectly adequate and may even be excessive. These are among the many questions that must be judiciously considered as we move forward into the next 5 years of this unprecedented molecular genetic screening program.

Key issues

- With the advent of professional recommendations for general population carrier screening, testing for cystic fibrosis mutations has rapidly become the highest volume molecular genetic test in many laboratories.
- Implementation of these recommendations has been supported by the development of robust technologies based on allele-specific DNA probe hybridization, oligonucleotide ligation, amplification refractory mutation detection, Invader assay and microarrays.
- While the analytical methods have been readily integrated into clinical molecular genetics laboratories, a number of pre- and postanalytical issues have proven problematic in offering, delivering, counseling and result reporting of these tests.
- Testing in large numbers of healthy people has revealed some unexpected genotype–phenotype relationships of certain cystic fibrosis transmembrane conductance regulator gene mutations and polymorphisms in the recommended screening panel.
- Over the next few years, further decreases in test cost and increased confidence of primary care physicians in the delivery of these tests should allow for accurate assessment of the program's effectiveness and societal acceptance.

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